

New Structural and Mechanistic Insights into Epigenetic Gene Regulation

Ming-Ming Zhou

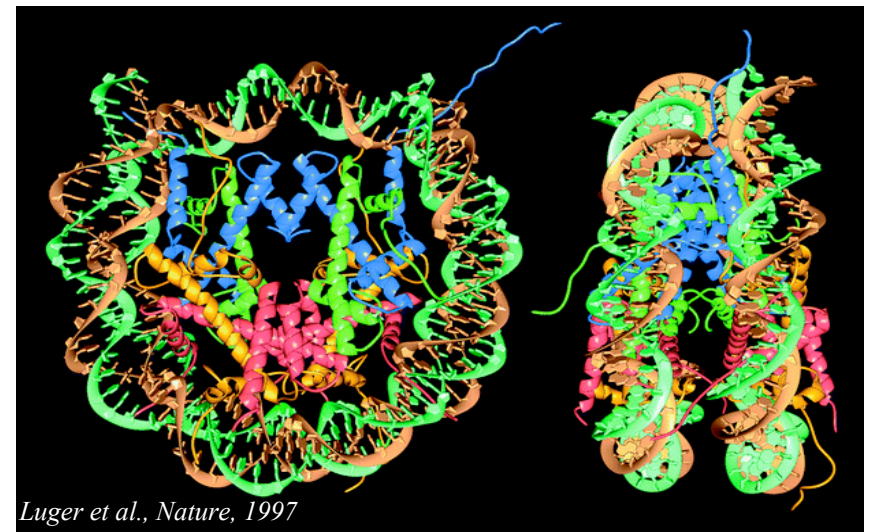
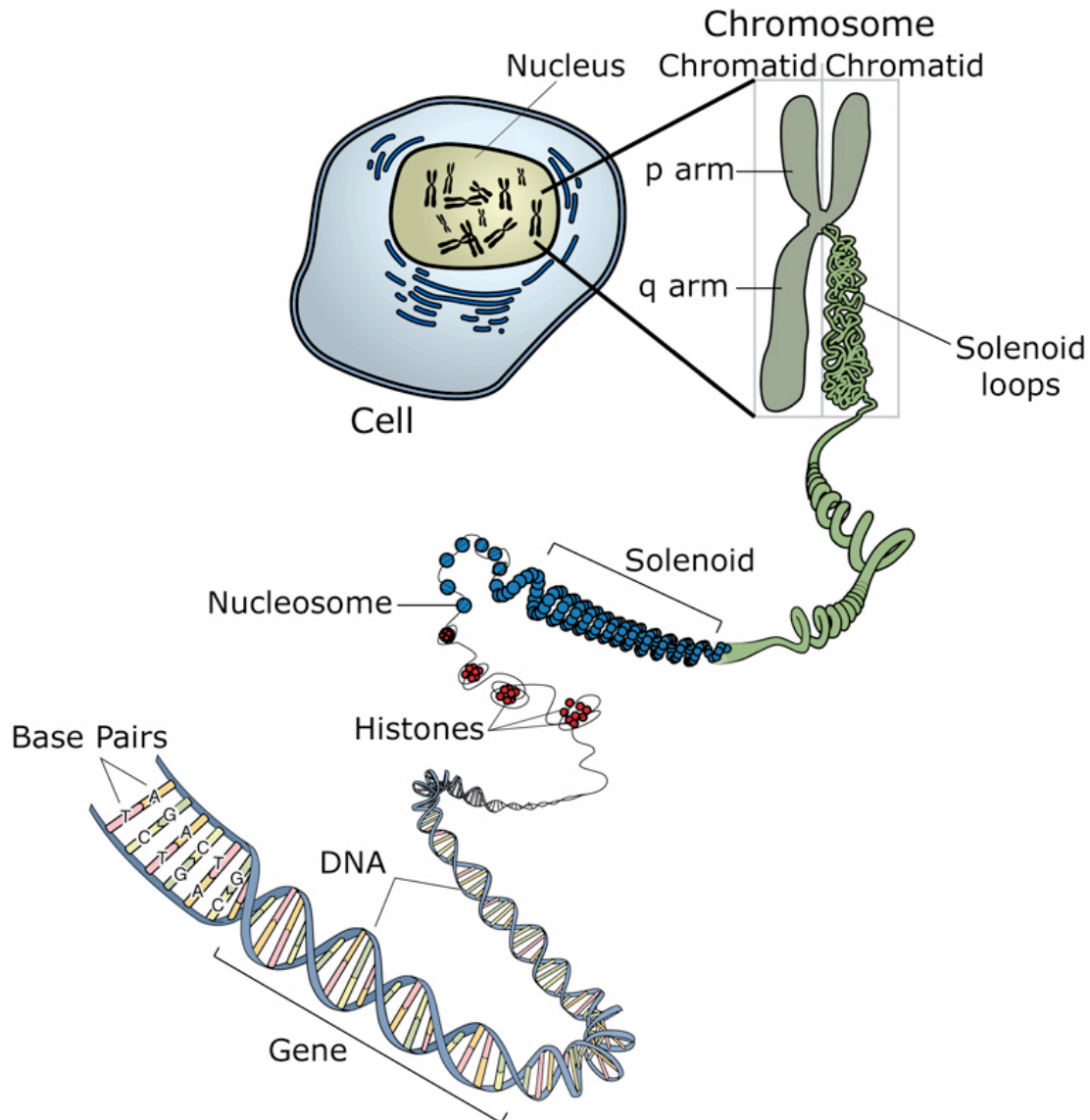
Mount Sinai School of Medicine
New York, NY

Brookhaven National Laboratory
February 23, 2009

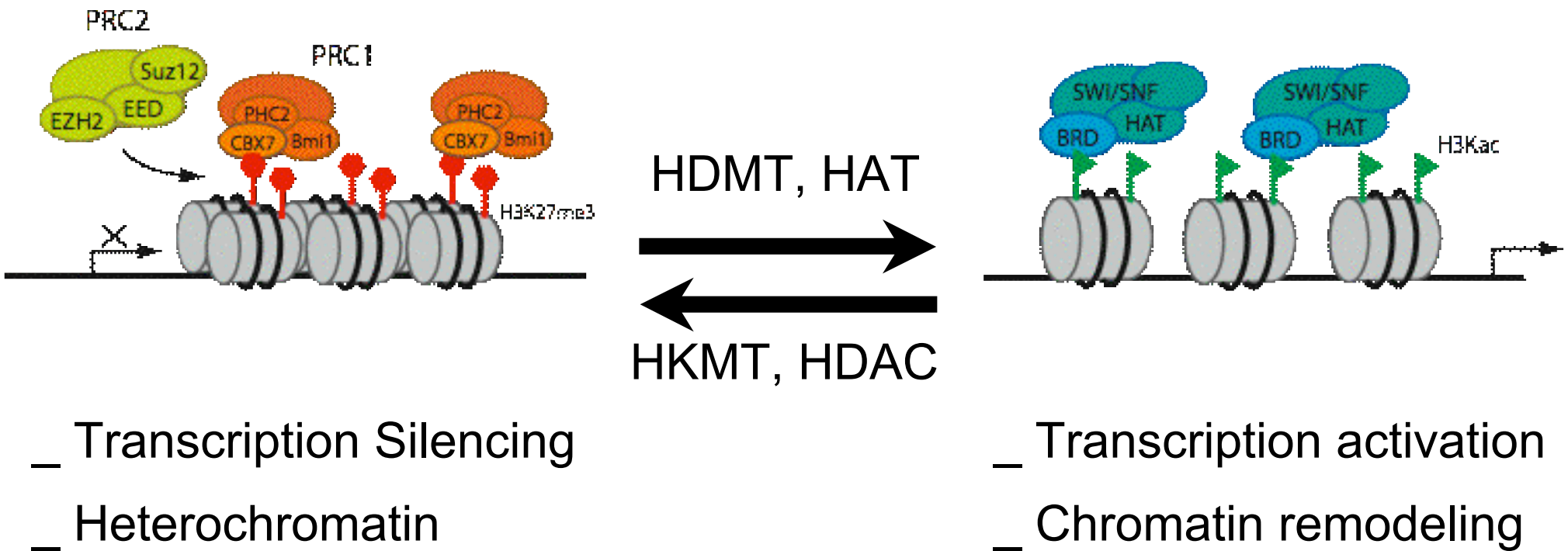


MOUNT SINAI
SCHOOL OF
MEDICINE

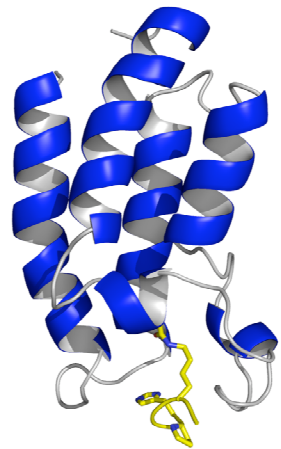
Chromatin and Epigenetic Gene Regulation



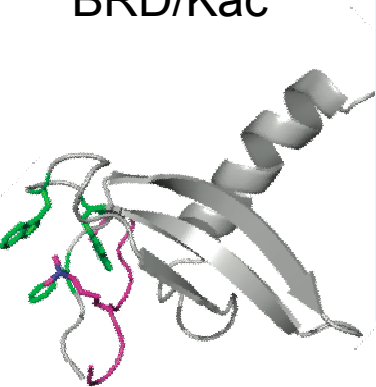
Epigenetic Control of Gene Transcription



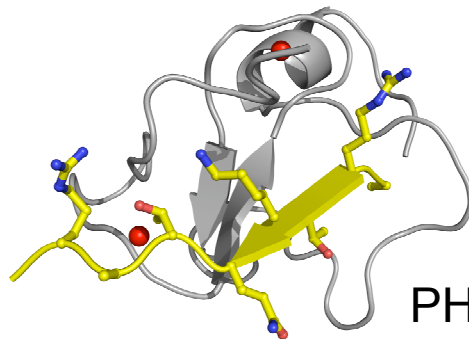
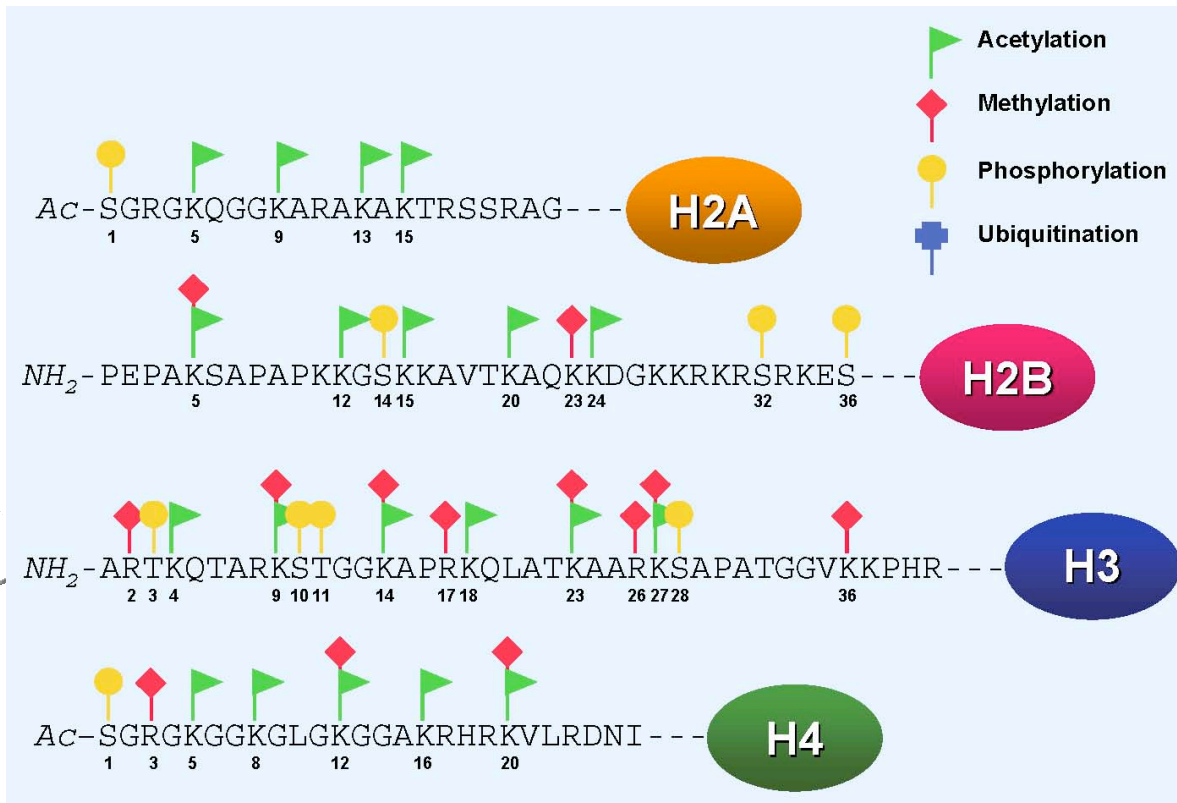
Histone Modifications and Interactions



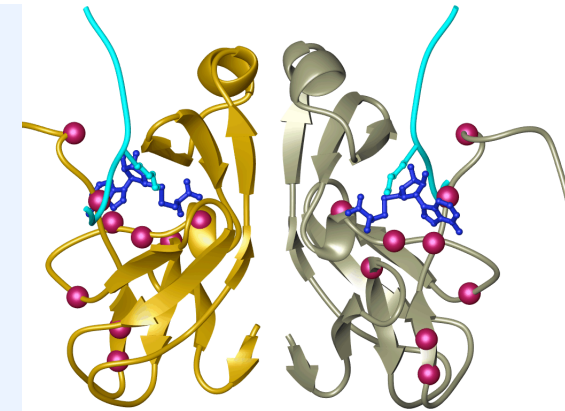
BRD/Kac



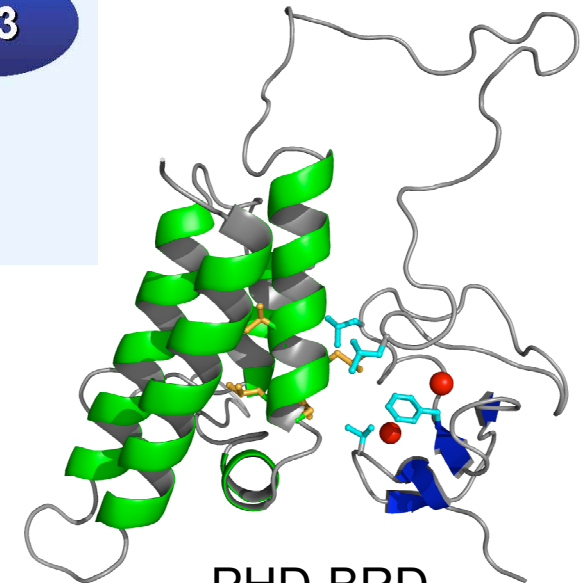
ChD/Kme



PHD/H3Kme0

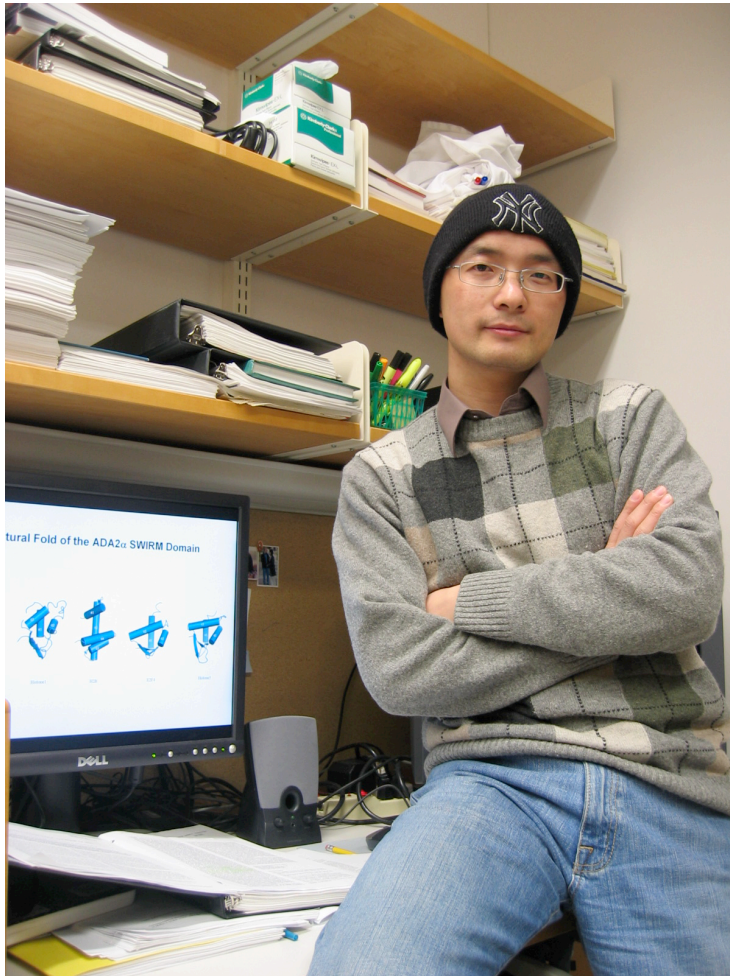


HKMTase



PHD-BRD

Structure and Function of the SRA Domain



Chengmin Qian, PhD

***Brookhaven National Laboratory
X6A Beamline
National Synchrotron Light Source***

Vivian Stojanoff, PhD
Jean Jakoncic, PhD

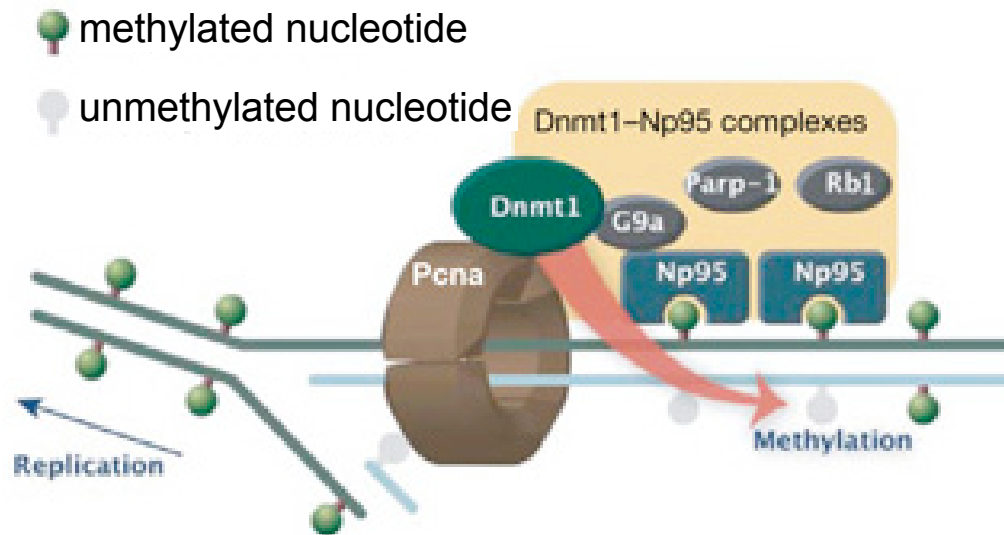
Mount Sinai School of Medicine

Martin Walsh, PhD
SiDe Li, PhD

Funding

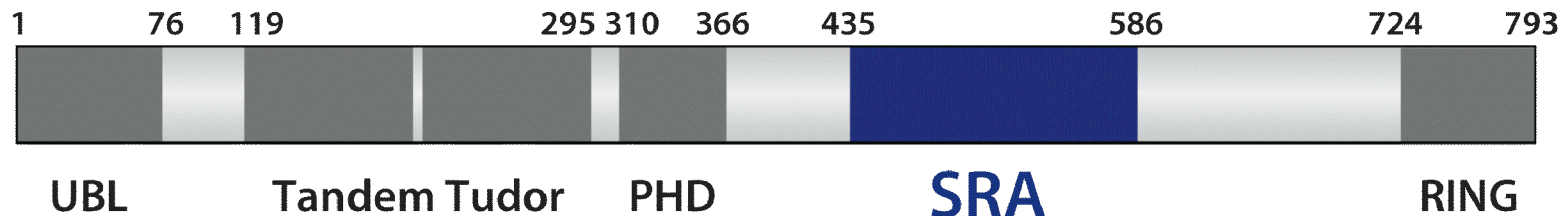
NIH / NIGMS, NCI
The New York Structural Biology Center

Crosstalk between DNA and Histone Methylation

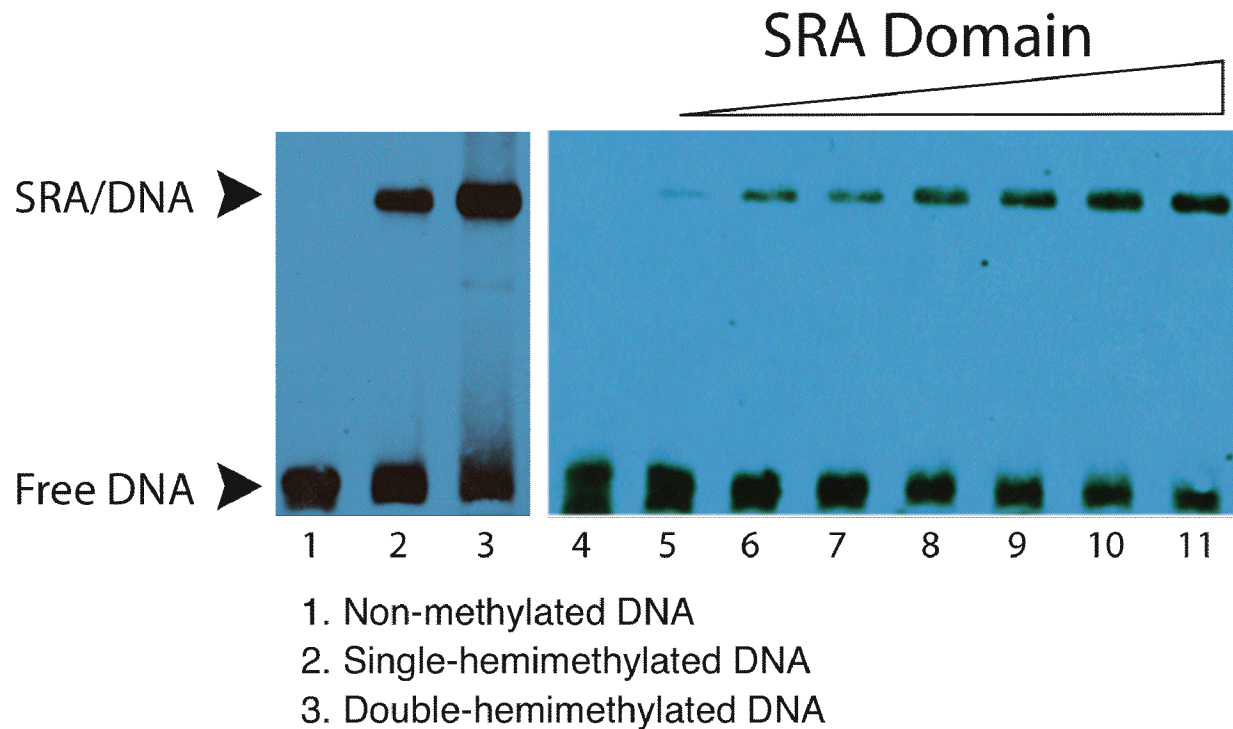


Sharif, J. et al., *Nature* (2007)

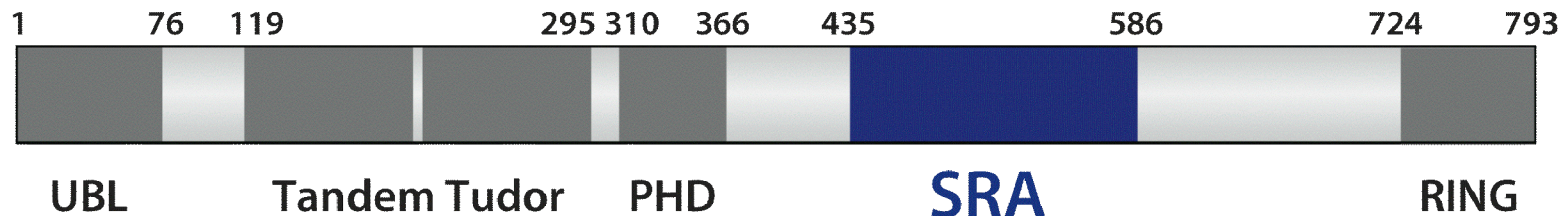
human UHRF1 (Np95/ICBP90)



Hemimethylated DNA Binding by the SRA Domain

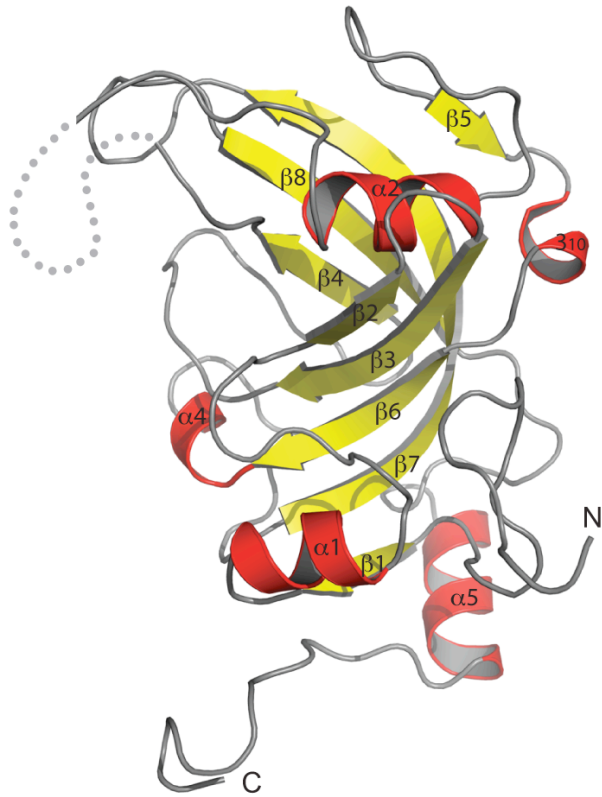


human UHRF1 (Np95/ICBP90)

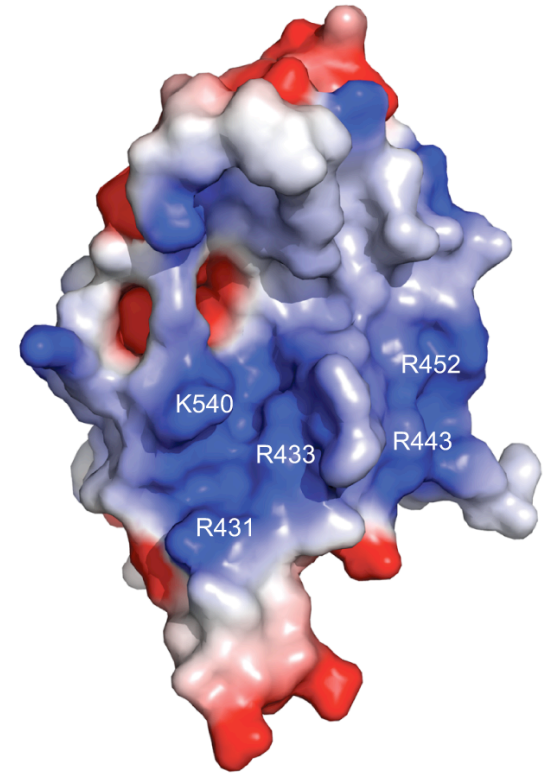
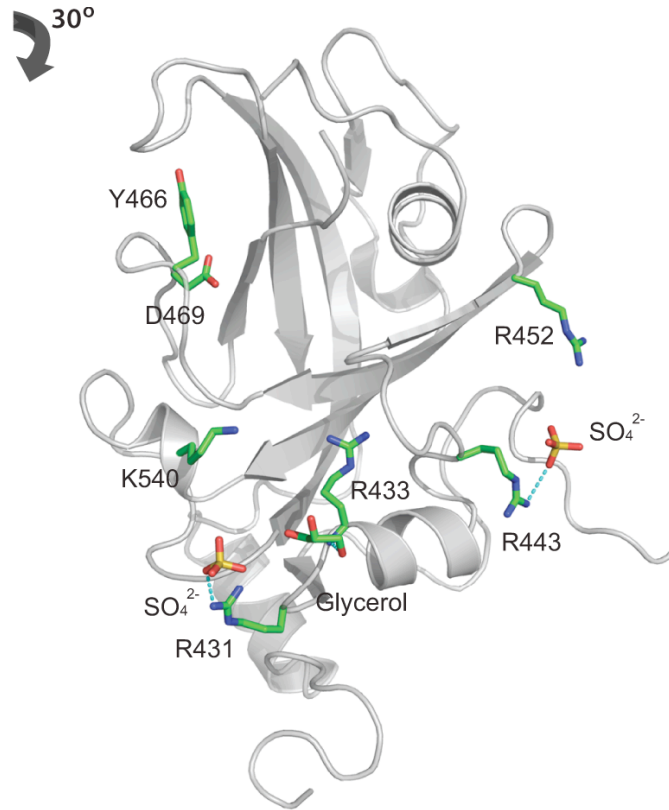


Crystal Structure of the Human UHRF1 SRA Domain

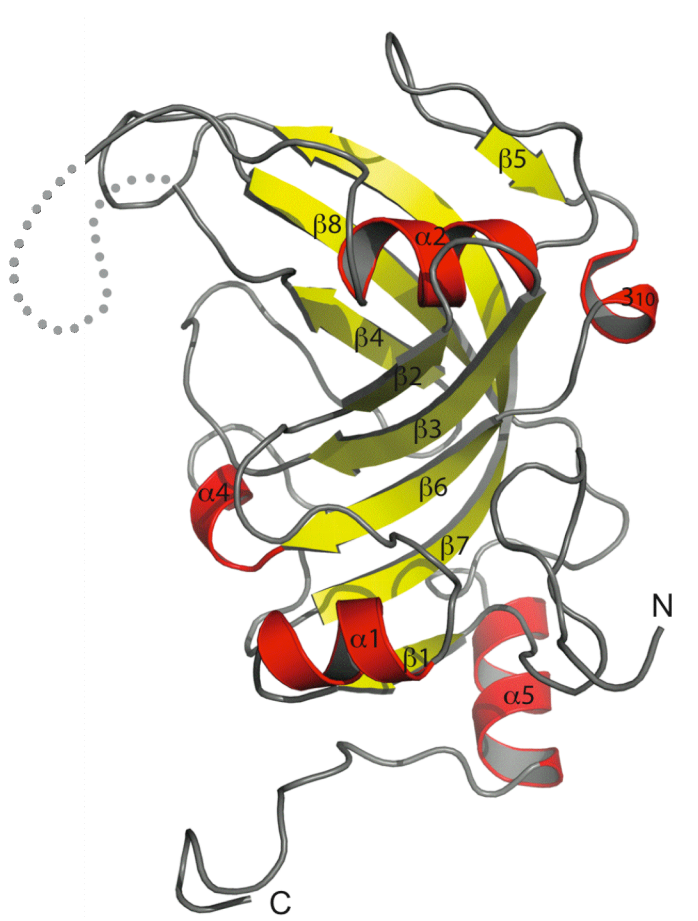
A



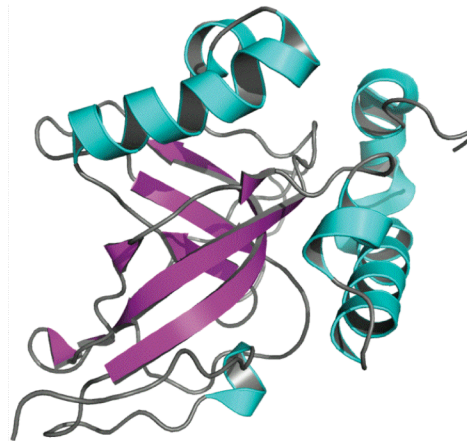
B



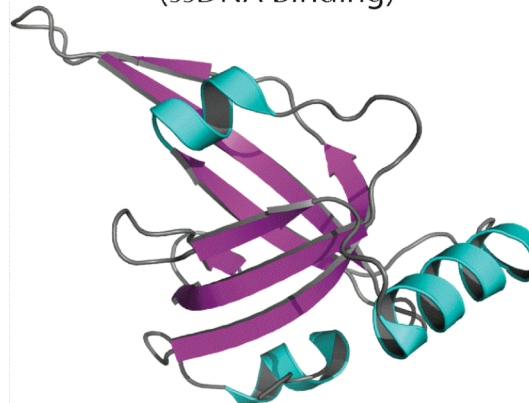
Structure Comparison of DNA Binding Domains



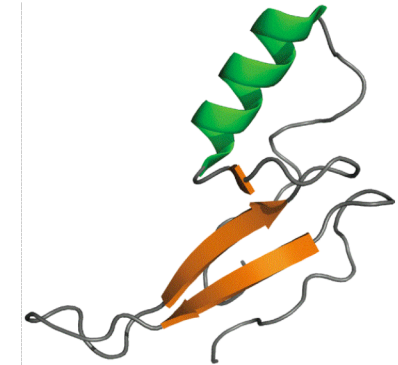
SRA Domain



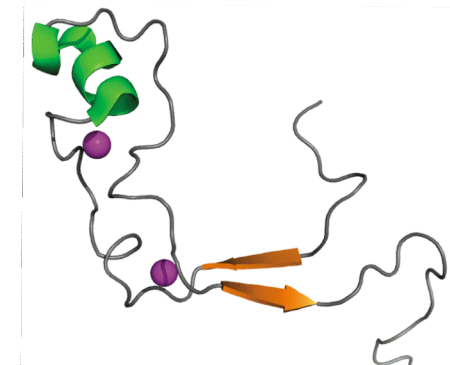
Cdc13
(ssDNA Binding)



RPA
(ssDNA Binding)



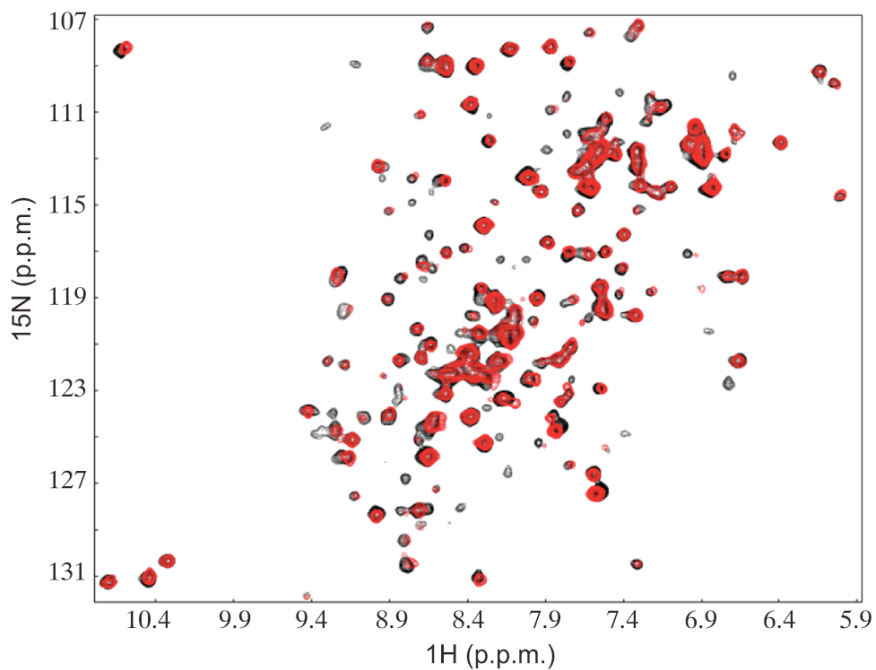
MeCP2
(methyl-CpG Binding)



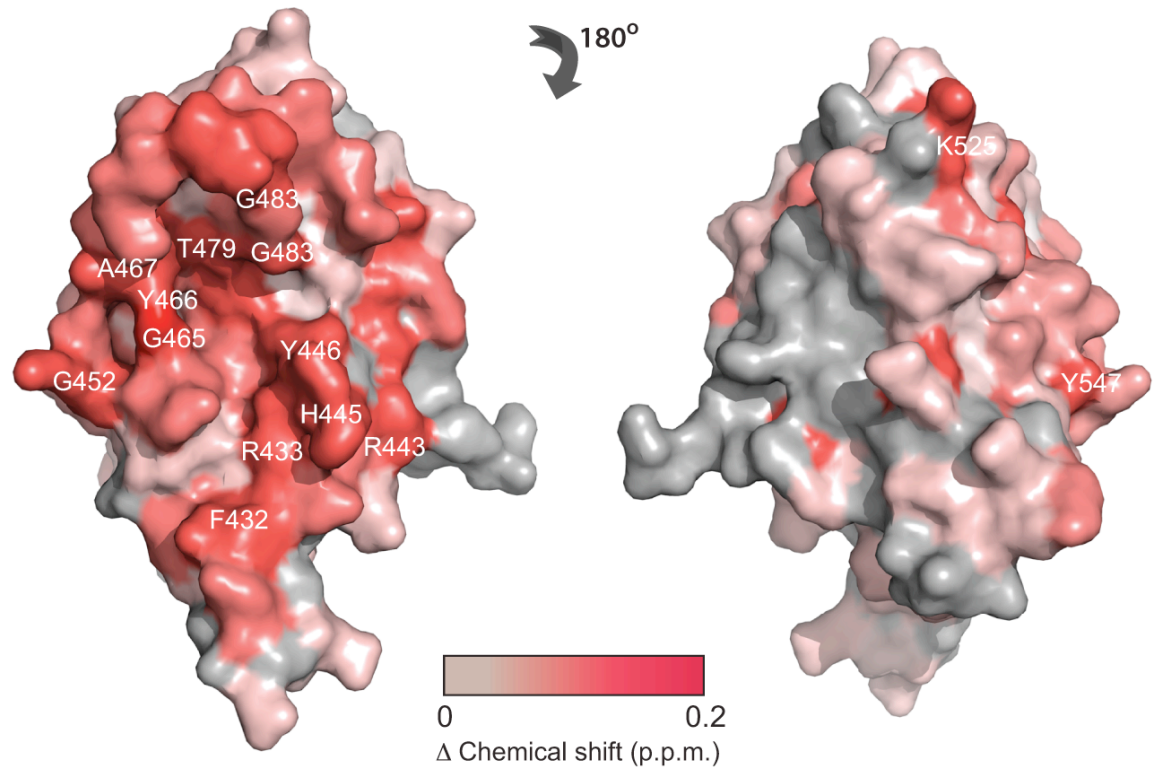
CXXC Domain
(non-methyl-CpG Binding)

Mapping of DNA Binding Site on the SRA Domain

A



B

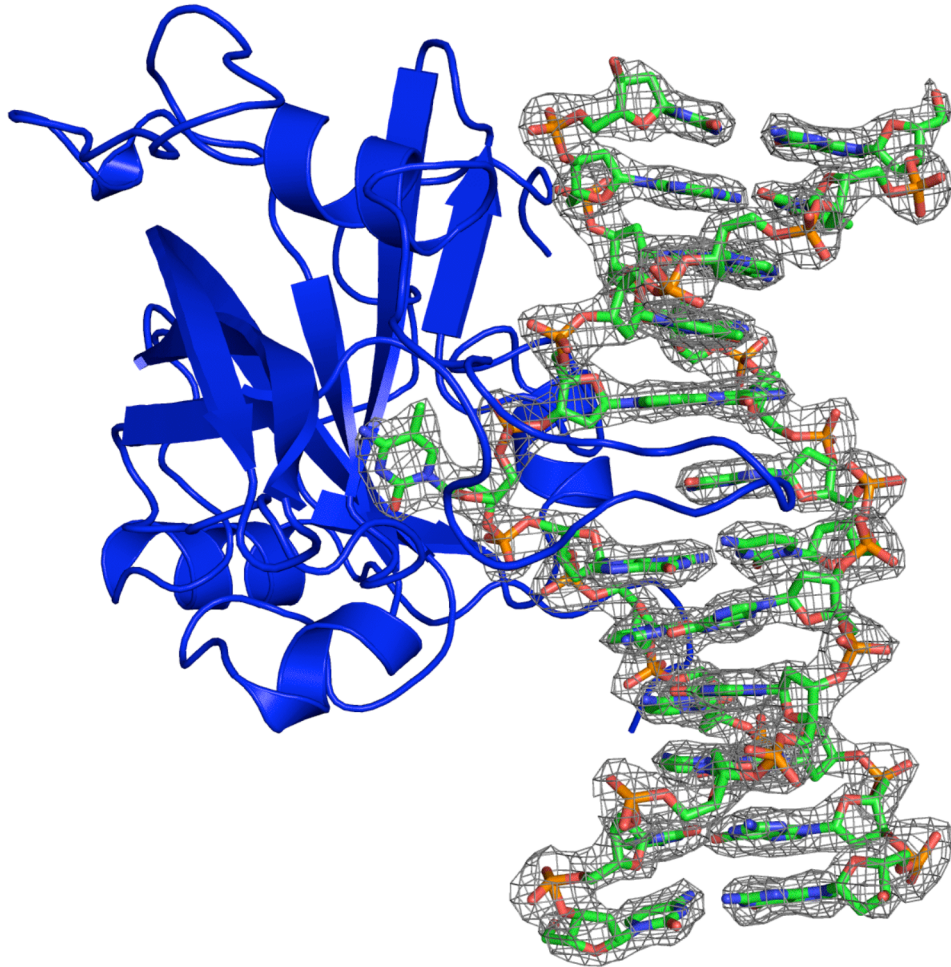


Crystallographic Statistics of Data Collection and Refinement

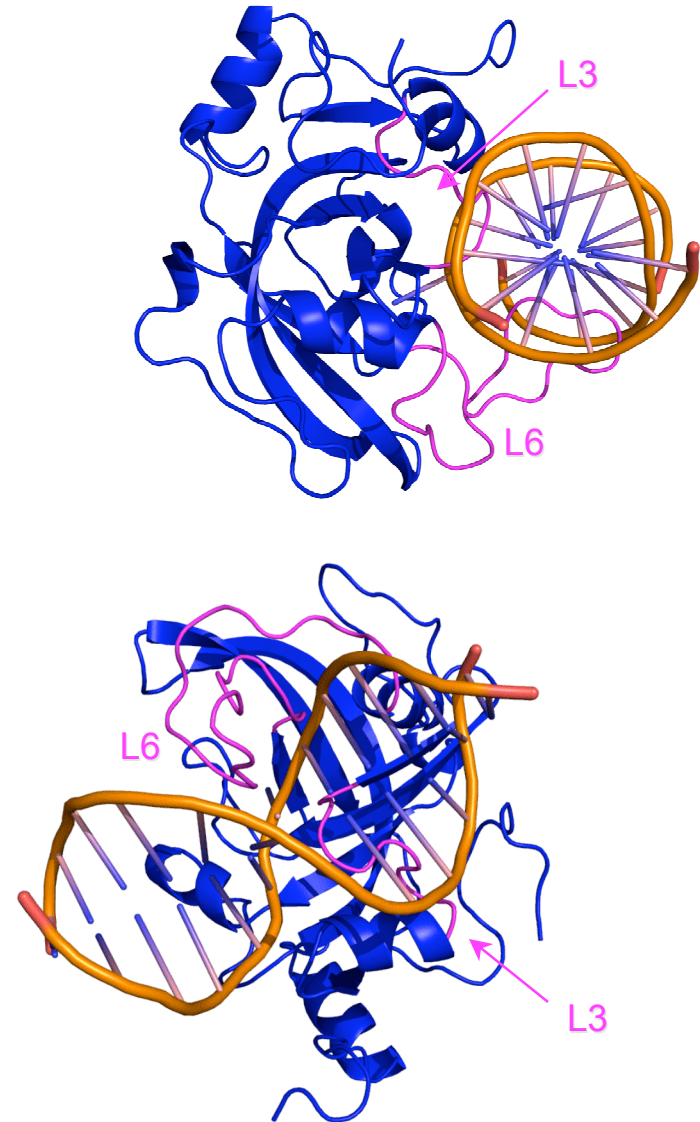
Data Collection	Free Form	Complex Form
Wavelength (Å) / Energy (keV)	0.9192 / 12.67	1.000 / 12.40
Space group	P3 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell Dimensions (Å)	a=65.56, b=65.56, c=95.36	a=78.70, b=91.26, c=95.86
Resolution Limits (Å)	20.00-1.95 (2.02-1.95)	25.00-2.45 (2.49-2.45)
Completeness (%)	98.7 (98.9)	98.9 (99.8)
Average I/ σ I	13.7 (2.5)	19.4 (2.6)
R _{merge} (%)	8.1 (35.3)	8.9 (48.3)
Average mosaicity (°)	0.35	0.70
Total reflections	53795	109680
Unique Reflections	17612 (1739)	26096 (1381)
Refinement Statistics		
Resolution (Å)	20.0-1.95 (2.00-1.95)	20.0-2.45 (2.50-2.45)
R _{cryst} / R _{free} (%)	19.9 / 25.3 (21.9 / 27.0)	21.4 / 27.3 (34.0 / 35.2)
Rms Deviations Bond length (Å) / angle (°)	0.017 / 1.608	0.011 / 1.966
non H atoms Protein/water/ligands	1662 / 135 / 2 SO ₄ + 1 Glycerol	4269/119
B (overall, prot, wat, lig) (Å ²)	19.9 / 19.4 / 24.8 / 26.3	56.3 / 56.9 / 54.7/ 51.0
Coordinates error DPI (Å)	0.152	0.272
Ramachandran plot quality Favored/Additional/Disallowed (%)	89.5 / 10.5 / 0.0	94.5/ 5.5/ 0.0

Structure of SRA Domain Bound to Hemimethylated DNA

A

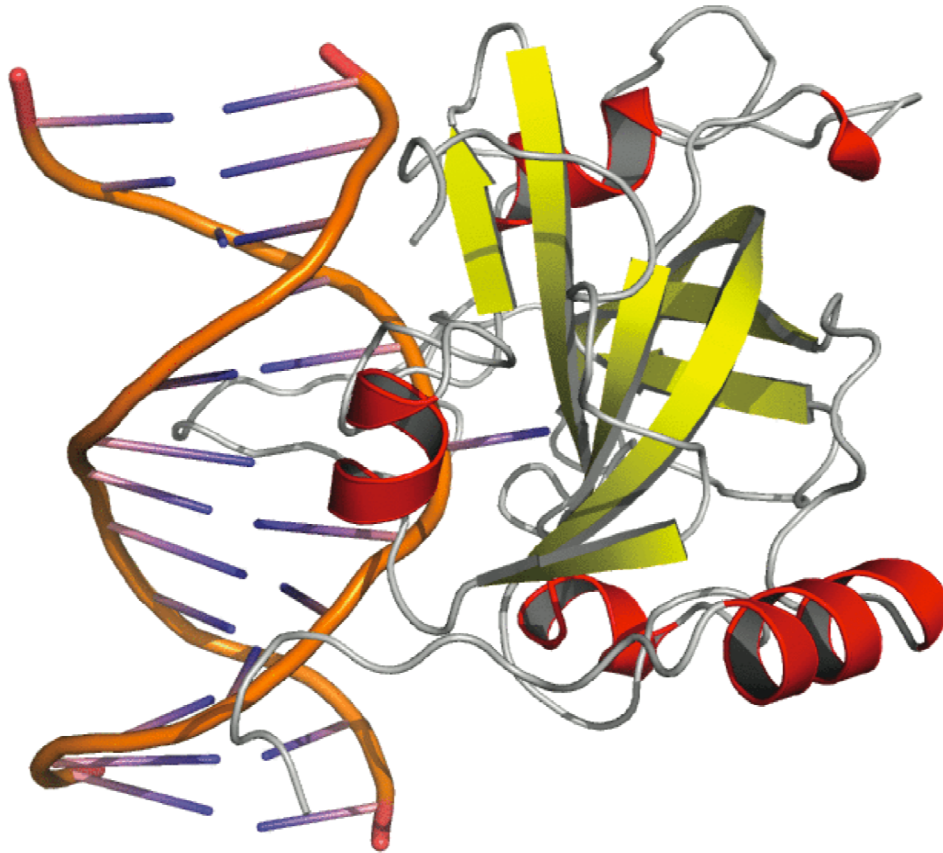


B

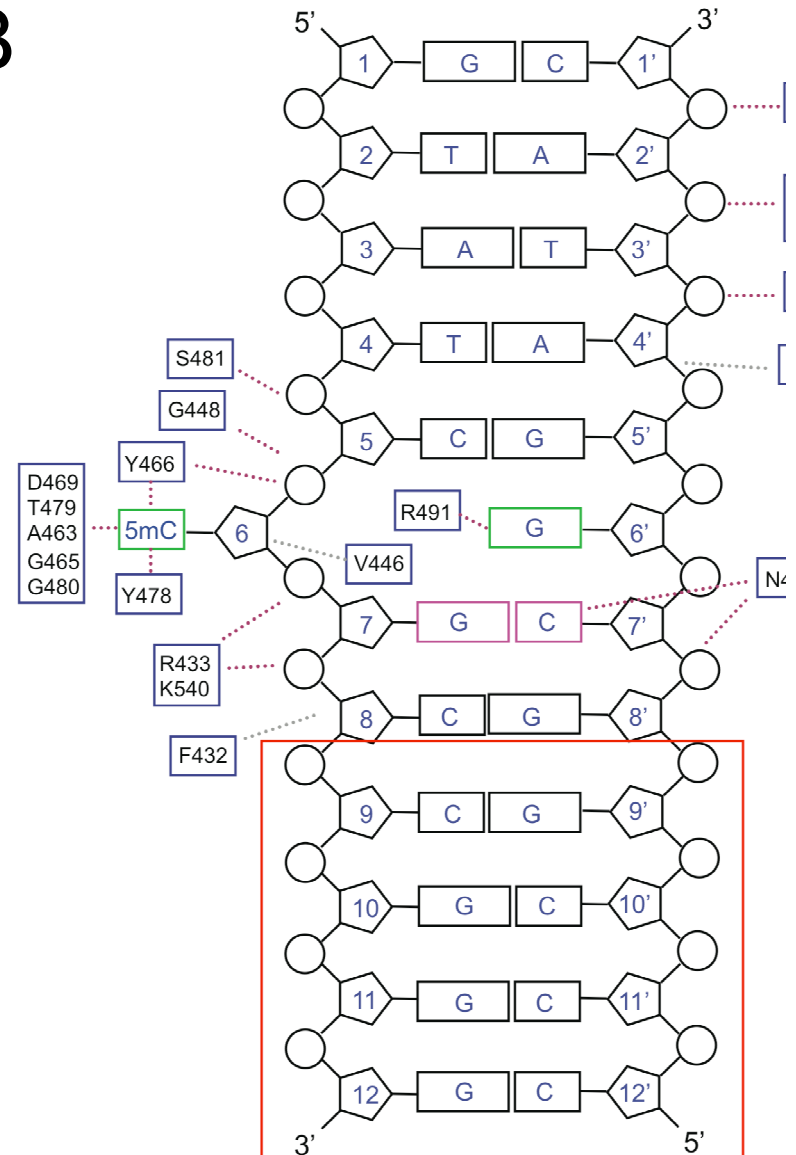


Hemimethylated CpG Recognition by the SRA Domain

A

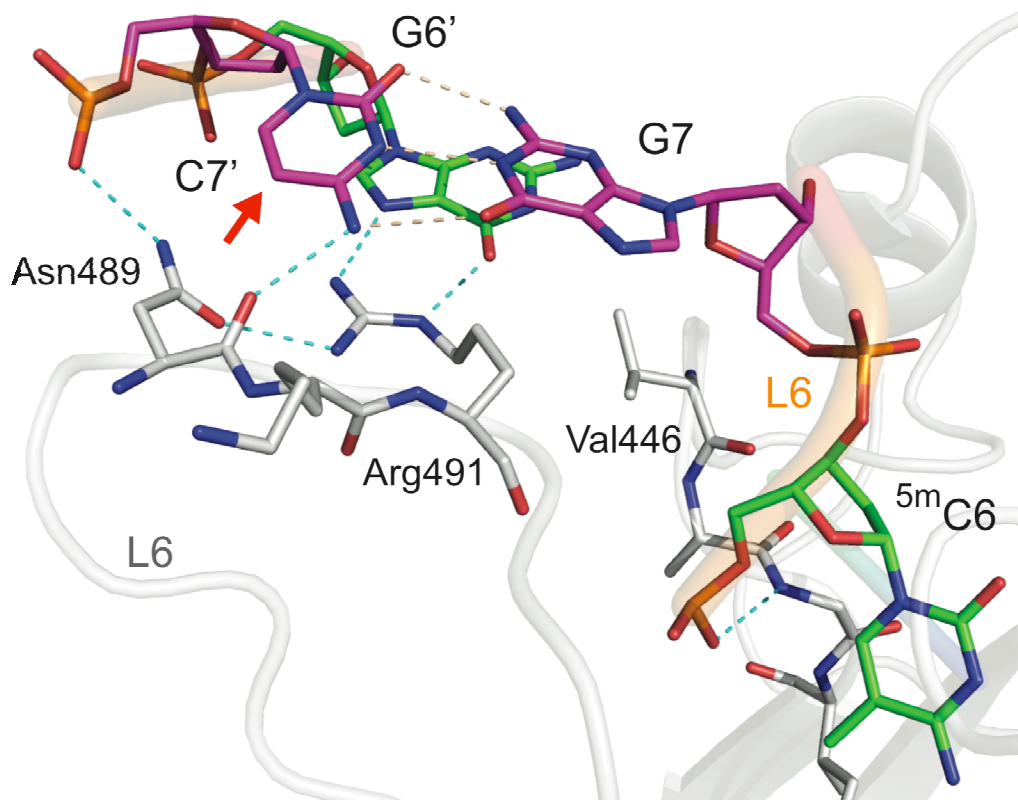


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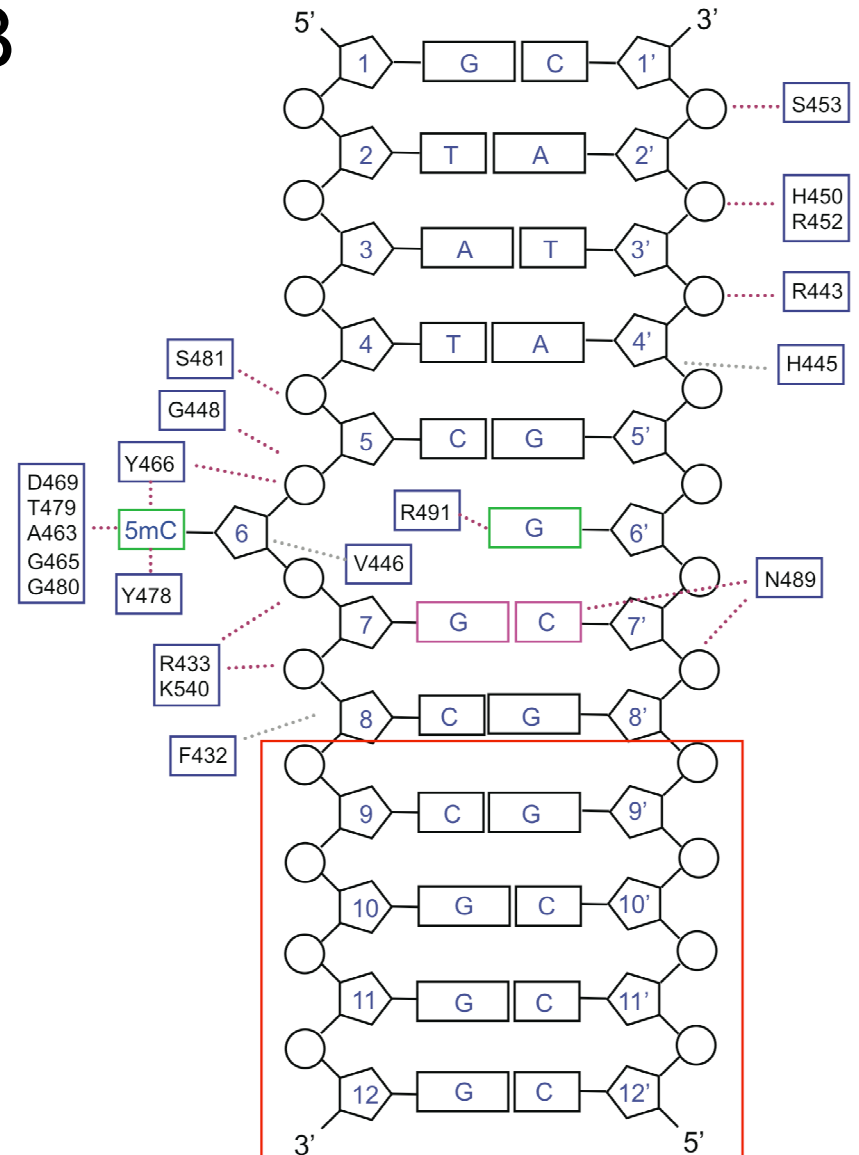


Hemimethylated CpG Recognition by the SRA Domain

A

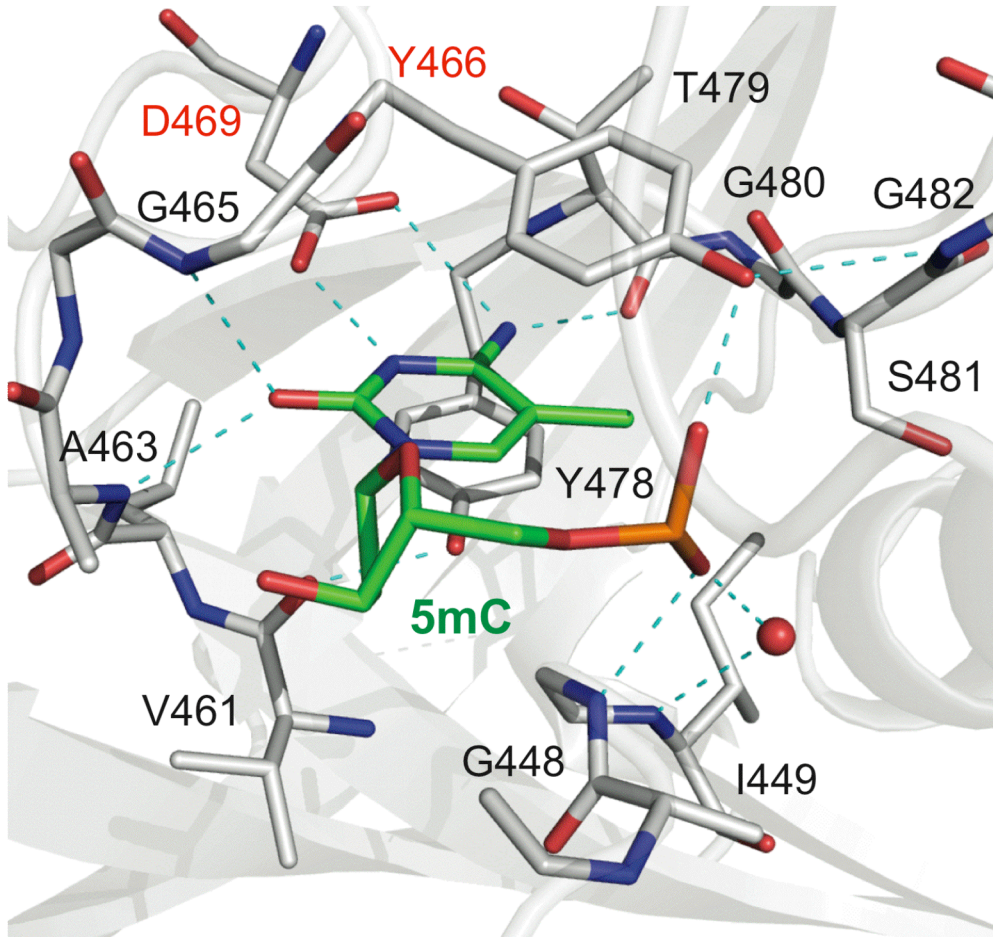


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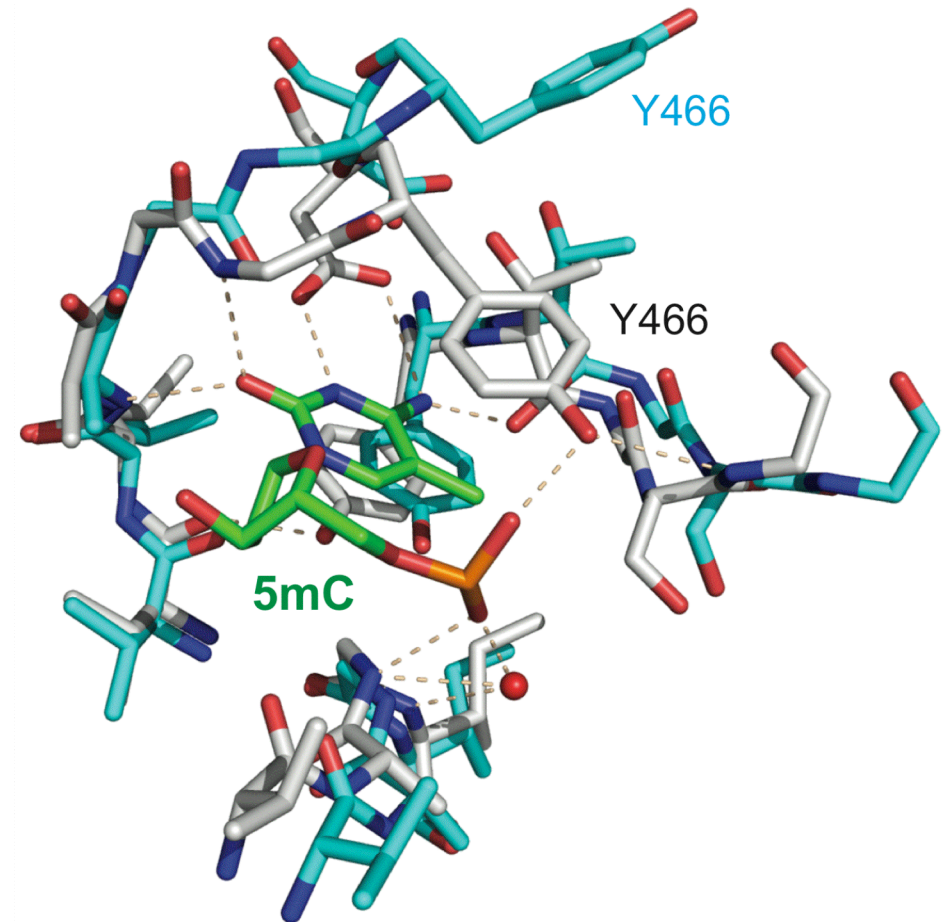
Hemimethylated CpG Recognition

A



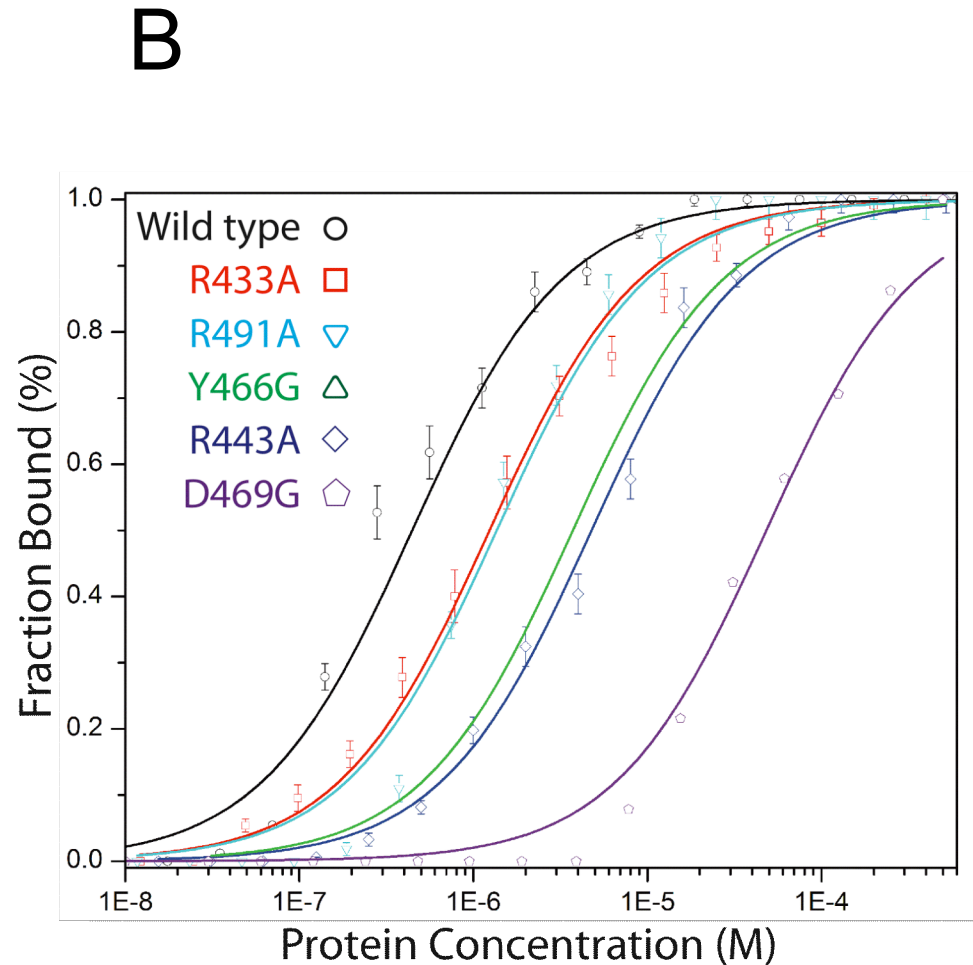
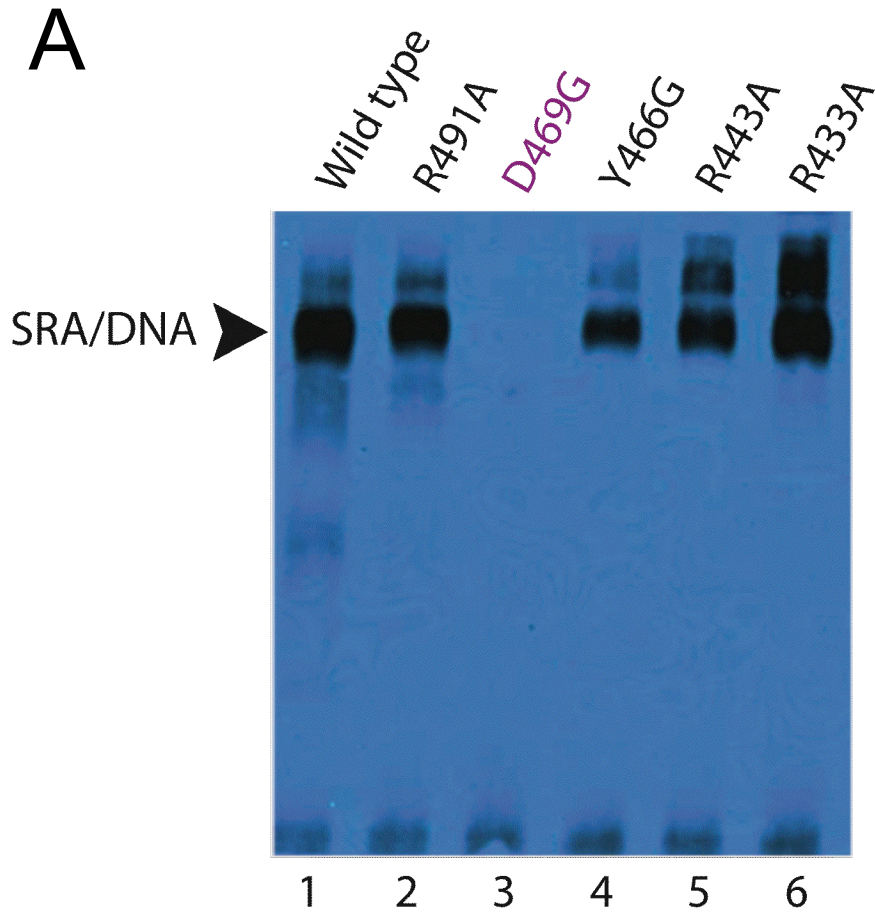
5mC base flipping recognition

B



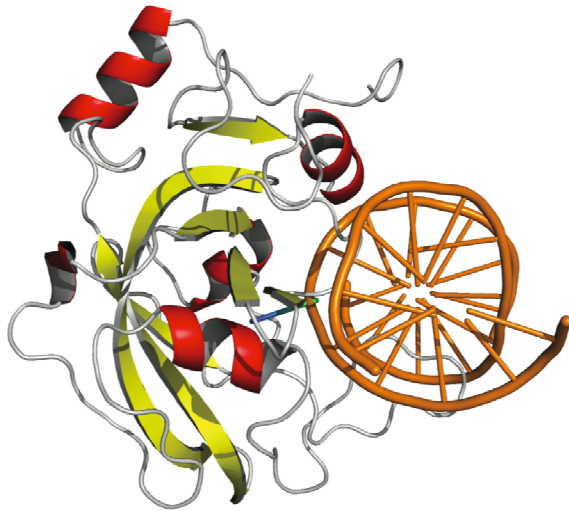
Structural rearrangement upon 5mC binding

Mutational Analysis of SRA Domain/DNA Binding

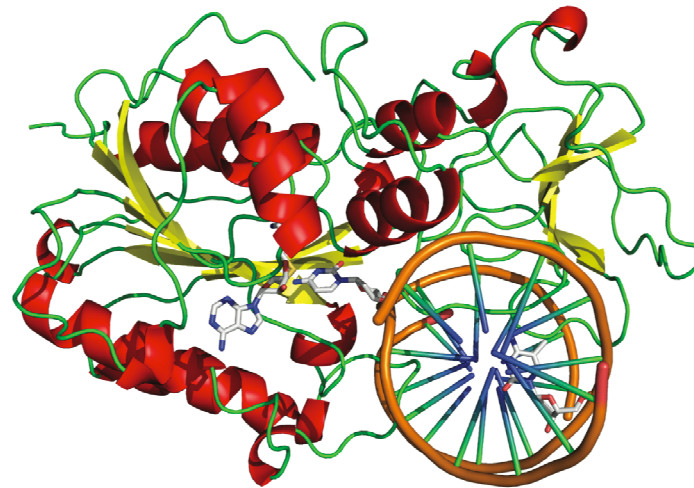


Base Flipping Mechanism for DNA Recognition

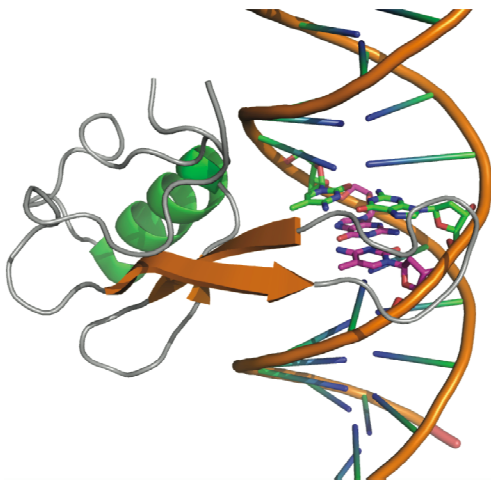
SRA



M.HhaI



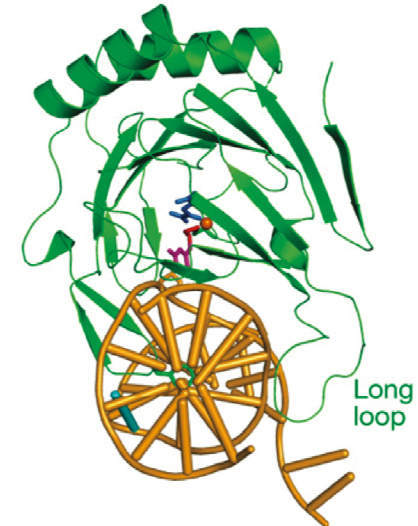
MeCP2



AlkB



ABH2



Summary

- ▶ The SRA domain structure is different that of MeCP2.
- ▶ Two loops interact with hemimethylated CpG duplex.
- ▶ Hemimethylated CpG recognition by a base-flipping mechanism.
- ▶ UHRF1 recognizes both methylated DNA and histone H3 in epigenetic gene regulation.

